BIOAVAILABILITY OF DRUGS AND BIOEQUIVALENCE

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INTRODUCTION

The U.S. Food and Drug Administration (FDA) defines *bioavailability* as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action" (1–3). Because in practice it is rare that drug concentrations can be determined at the site of action (e.g., at a receptor site), bioavailability is more commonly defined as "the rate and extent that the active drug is absorbed from a dosage form and becomes available in the systemic circulation." Usually bioavailability refers to the absorption of a drug from the gastrointestinal tract following oral administration of a dosage form. The dosage form may be of any type, including a solution, suspension, tablet, capsule, powder, or elixir. Bioavailability can also refer to the absorption of a drug from other routes of administration, such as intramuscular (IM) injection, transdermal patches, ointments and other topical preparations, and implants, which also require absorption prior to reaching the systemic circulation. As these routes of administration (e.g., oral, IM, and topical) deliver the drug to a site outside the vascular system, they are often referred to as routes of extravascular administration. The only route of drug administration that will always result in a bioavailability of 100% is an intravenous injection, in which the amount of drug reaching the systemic circulation is equal to the total administered dose.

The term *relative bioavailability* refers to a comparison of two or more dosage forms in terms of their relative rate and extent of absorption. If an intravenous injection is employed as the reference dose, one can determine the *absolute bioavailability* of the test dosage form. Two dosage forms that do not differ significantly in their rate and extent of absorption are termed *bioequivalent*.

In general, bioequivalence evaluations involve comparisons of dosage forms that are *pharmaceutical equivalents*. Such dosage forms are defined as "drug products that contain identical amounts of the identical active drug ingredient, i.e., the same salt or ester of the same therapeutic moiety, in identical dosage forms, but not necessarily containing the same inactive ingredients, and that meet the identical compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates" (1–3). Bioequivalence determinations may also be made for *pharmaceutical alternatives*, defined as "drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester. Each such drug product individually meets either the identical or its own respective compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates." In some instances, two pharmaceutical alternatives exhibit markedly different bioavailability, for example, a rapidly absorbed elixir versus a more slowly absorbed capsule. In other cases, two different dosage forms (e.g., a tablet and a capsule) may or may not exhibit very similar bioavailability.

FACTORS AFFECTING DRUG BIOAVAILABILITY

Extravascularly administered drugs must traverse several barriers to reach the systemic circulation and/or their site of action. Many studies illustrate that differences in manufacturing procedures, as well as the composition of the dosage form, can affect the bioavailability of a drug product. In addition, the bioavailability of a drug product can also be influenced by the physiology of the patient and other factors, such as the content of the gastrointestinal tract. The steps involved in the disposition of an orally administered solid dosage form and the experimental approaches that may be used to characterize them are summarized in Table 1. Factors affecting the bioavailability of a dosage form, discussed briefly here, are discussed in detail elsewhere in this encyclopedia.[a]

[a]See *Absorption of Drugs*, page 8; *Biopharmaceutics*, page 156.

Table 1 Disposition and evaluation of orally administered solid dosage forms

Steps involved in disposition	Experimental approaches
Dosage form reaches stomach/intestine	Measurement of pH or scintigraphy
Dosage form disintegrates into small particles[a]	In vitro disintegration testing
Drug dissolves in gastrointestinal fluids	In vitro dissolution testing
Drug reaches gastrointestinal wall/membrane	
Drug is returned to gastrointestinal lumen by P-glycoprotein efflux pump	In vitro drug transport studies
Drug is metabolized by intestinal enzymes	In vitro drug metabolism studies
Drug is absorbed into hepatic circulation	In situ/in vivo hepatic perfusion studies
Drug reaches systemic circulation	Assay of drug in blood, plasma, or serum
Drug is excreted in urine	Measurement of drug excretion in urine
Drug is metabolized	Measurement of metabolite(s) in blood and/or urine
Drug and/or active metabolite reaches its site of action and causes a pharmacologic response	
Response is unrelated to desired therapeutic activity	Measurement of onset, duration, and intensity of pharmacologic response
Response related to desired clinical response	Determination of clinical efficacy in patients

[a]Some dosage forms are designed to remain intact (e.g., certain controlled-release products).

A major factor determining the bioavailability of an orally administered drug product is the dissolution rate of the drug. A drug must be in solution in order to be absorbed from the gastrointestinal tract. Even if the drug product is administered as a solution, some dissolution process may be required in the event that the drug precipitates as a result of low solubility in the fluids of the gastrointestinal tract.

Drug Product Formulation

Most drugs are not taken as pure chemicals but are formulated into a pharmaceutical dosage form. Such drug products may be a relatively simple solution or a compressed tablet containing binders, fillers, lubricants, a coloring agent, and the like; or a controlled-release product. The following are some of the formulation and manufacturing variables that could influence the bioavailability of a drug product: 1) the properties of the drug (salt form, crystalline structure, formation of solvates, solubility); 2) the composition of the finished dosage form (presence or absence of excipients, special coatings); 3) manufacturing variables (tablet compression force, processing variables, particle size of drug or excipients, environmental conditions); and 4) rate and/or site of dissolution in the gastrointestinal tract.

Physiologic and Other Factors Affecting Bioavailability

The rate and extent of drug absorption can also be affected by a wide variety of factors related to the characteristics of the subject/patient receiving the drug product. These factors are important to consider because they can contribute to intrasubject and intersubject variability during treatment, and, if not well controlled during the course of a bioavailability study, they can bias the results and confound interpretation of the data. Examples include: 1) contents of the gastrointestinal tract (fluid volume and pH, diet, presence or absence of food, bacterial activity, presence of other drugs); 2) rate of gastrointestinal tract transit (influenced by disease, physical activity, drugs, emotional status of subject, and composition of the gastrointestinal tract contents); 3) pre-systemic drug metabolism and/or degradation (influenced by local blood flow; condition of the gastrointestinal tract membranes; drug transport, metabolism or degradation in the gastrointestinal tract or during the first pass of the drug through the liver); 4) age, sex, race, disease, body size, time of day, and physical activity.

Other factors related to the subject, if not recognized or controlled, can also influence the assessment of drug bioavailability and product bioequivalence. For example, bioavailability studies typically involve the collection of blood and/or urine specimens to determine drug appearance in the systemic circulation. Thus, physiologic and pharmacokinetic perturbations that affect the concentration of drug measured in these fluids can potentially influence the results and interpretation of the study. Examples include changes that alter 1) the rate, extent, and/or route of metabolism (e.g., coadministered drugs that compete for or induce drug metabolizing

enzymes); 2) the rate and/or extent of drug elimination by the kidney (e.g., kidney disease and/or competition and changes in urine pH that affect renal transport mechanisms); 3) the degree of binding of the drug to plasma or tissue proteins (e.g., age-related changes in plasma binding proteins or protein binding displacements); and 4) distribution of drug into the erythrocytes. Genetic polymorphisms in the drug metabolizing enzymes of the liver may also contribute to large differences in the pharmacokinetics of a drug and the interpretation of bioavailability studies (4). A well-designed bioavailability study must either control or account for the influence of such variables.

CHARACTERISTICS OF DRUGS WITH THE GREATEST POTENTIAL FOR A BIOAVAILABILITY PROBLEM

The total number of marketed drug products known to exhibit a significant bioavailability problem is relatively small. Thus, one view is that bioavailability has been overemphasized and that for most drug products it is not a matter of concern. Another view is that those products that exhibit a bioavailability deficiency in a carefully controlled study provide ample evidence of the potential of a bioavailability problem for many drug products not yet studied.

With minor exceptions, the U.S. FDA has required that bioavailability and bioequivalence of a drug product be demonstrated through in vivo studies. However, a Biopharmaceutics Classification System (BCS) was recently proposed that divides drugs into classes based on their solubility, permeability, and in vitro dissolution rate (5). This classification system could be used to justify the waiver of the requirement for in vivo studies for "rapidly dissolving drug products containing active moieties/active ingredients that are highly soluble and highly permeable." If adopted by the FDA, the bioavailability and bioequivalence of drug products meeting these requirements could be demonstrated using in vitro solubility, permeability, and dissolution studies. However, drugs that are poorly permeable, poorly soluble, and/or formulated in slowly dissolving dosage forms would be considered more likely to demonstrate a bioavailability problem and thus, would not be candidates for the waiver of in vivo bioavailability studies.

The U.S. FDA published a summary of evidence that may be employed to assess the importance of establishing the bioavailability of a given drug (3). The summary is as follow:

1. Data from clinical trials or bioequivalence studies indicate a bioequivalence problem.
2. The drug has a narrow therapeutic ratio, and the drug concentrations in the patient must be carefully adjusted.
3. A lack of bioequivalence could have serious medical consequences.
4. Physicochemical evidence indicates that:
   a. the drug has low solubility in water and/or the dissolution rate of the dosage form is slow;
   b. the particle size, crystalline structure, and other factors of the drug can affect the dissolution and bioavailability; and
   c. the drug product contains a high ratio of excipients to active ingredients, or the product may require excipients to enhance absorption or contain excipients that inhibit absorption.
5. Pharmacokinetic evidence indicates that:
   a. the absorption of the active drug is limited to a specific region of the gastrointestinal tract;
   b. the extent of absorption is low;
   c. there is rapid metabolism such that rapid dissolution and absorption are required for effectiveness;
   d. the product required special formulations to stabilize the drug in the gastrointestinal fluids; and
   e. the drug exhibits dose-dependent pharmacokinetics.

Drugs that meet one or more of the above criteria and have been shown to exhibit significant differences in the bioavailability of marketed dosage forms include: digoxin, quinidine, furosemide, nitrofurantoin, prednisone, chloramphenicol, theophylline, chlorpromazine, phenytoin, amitriptyline, and phenylbutazone.

EXPERIMENTAL DETERMINATION OF BIOAVAILABILITY

Types of Studies

Several methods can be used to determine the bioavailability or bioequivalence of a drug product. The vast majority of bioavailability studies involve the administration of the test dosage form to a group of healthy human subjects, followed by the collection and assay of the drug concentration in blood (plasma or serum) samples. The second most frequent method utilizes urinary excretion measurements. Occasionally, other types of biological

material, such as saliva, cerebrospinal fluid, bile, or feces are also collected. For a few drugs, for which assay methods are not available for the determination of drug concentrations in biological fluids, a pharmacologic response may be measured. Finally, some bioavailability assessments have been made on the basis of a determination of the therapeutic response of patients to a given dosage form. However, this type of study is usually restricted to drugs that are active at the site of administration (e.g., topical) but are not intended to be available in the systemic circulation. For approval by the U.S. FDA, pharmacokinetic, pharmacodynamic, clinical, and in vitro studies are recognized (in descending order of preference) as acceptable approaches to document the bioavailability or bioequivalence of a drug product.

Blood level studies

The primary basis for blood concentration studies is the assumption that two dosage forms that exhibit superimposable blood concentration time profiles in a group of subjects should result in identical therapeutic activity in patients. Figure 1 illustrates a theoretical blood concentration time curve after the oral administration of a drug product. The key parameters to note from this figure are the maximum blood concentration (C_{max}), the time (T_{max}) of occurrence of the maximum blood concentration, and the total area under the blood concentration time curve (AUC). The value of T_{max} provides a means to assess the rate of absorption of the drug. The T_{max} is independent of the amount of drug absorbed but is inversely related to the absorption rate. Thus, the faster the absorption of a drug, the shorter will be the T_{max}. The value of T_{max} is also influenced by the rate of elimination of the drug from the body. However, if one assumes elimination rate does not change during the period when two or more dosage forms are being tested in a given subject, then observed differences in T_{max} will reflect absorption rate differences among the test products. The interpretation of C_{max} is somewhat more complicated because it is a function of both the rate of absorption and the extent of absorption, as well as the elimination rate. Thus, as the amount of the drug absorbed increases and/or the rate of absorption increases, the C_{max} also increases, assuming no change in elimination rate. A determination of the extent of drug absorption is usually based on a measure of AUC, which is directly proportional to the fraction of the administered dose that reaches the systemic circulation and is independent of the rate of absorption. The calculation of AUC is commonly accomplished using the linear trapezoidal rule (Fig. 1). The blood concentration time curve is divided into a series of geometric sections, and the

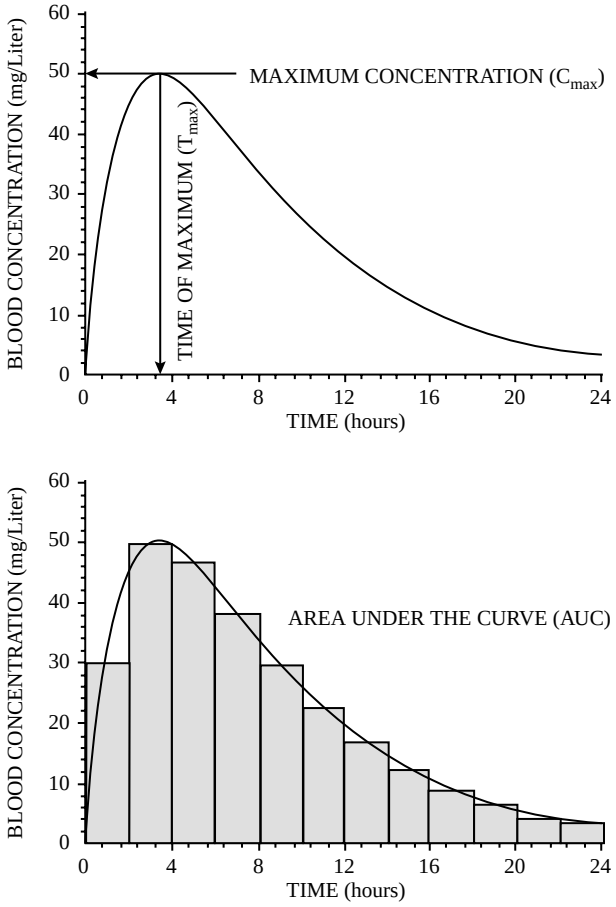

Fig. 1 Drug concentration in blood time plots illustrating calculation of C_{max}, T_{max}, and AUC.

area encompassed by each section is determined from the trapezoidal rule

$$\mathrm{AUC} = 12 * (\Delta t) * (C_1 + C_2) \quad (1)$$

where Δt is the time interval between the collection of two blood samples of concentrations C_1 and C_2. The units of AUC are given as the product of concentration and time (e.g., mg/Liter × hr). If blood samples are not obtained for a sufficient period of time to result in a zero drug concentration in the final sample, it is necessary to estimate the portion of the AUC remaining after the final sample. Eq. 2 gives the relationship between the AUC $(0 - t)$ for the portion of the curve to the last sample taken at time t, and the total AUC$(0 - \infty)$

$$\mathrm{AUC}(0-\infty) = \mathrm{AUC}(0-t_{\mathrm{last}}) + C_{\mathrm{last}}/K \quad (2)$$

where C_{last} is the last measurable drug concentration, t_{last} is the time at which it was collected, and K is the apparent first-order elimination rate constant estimated

from the terminal slope of the log-linear plot of concentration versus time. In studies involving blood sampling intervals after the peak that are relatively long compared with the half-life of the drug, the use of the logarithmic trapezoidal method has been recommended to estimate the postpeak AUC (6).

The value of AUC($0 - \infty$) may also be as expressed in Eq. 3:

$$\text{AUC}(0-\infty) = F * D/CL \tag{3}$$

where F is the fraction of dose absorbed, D is the administered dose, and CL is the total body clearance of the drug. Thus, a comparison may be made between two different dosage forms on the basis of the ratio of their respective AUC($0 - \infty$) values. Assuming equivalent doses are given and the clearance of the drug remains constant during the time the two doses are administered, the ratio of the AUCs will be directly proportional to the ratio of the fraction of each dose absorbed, that is, the extent of absorption.

Figure 2 illustrates the types of plasma concentration time data that might be obtained during the testing of three different bioinequivalent formulations of a drug. Products A and C are absorbed at the same rate, based on identical T_{max} values, but B is more slowly absorbed, as shown by the longer time required to achieve C_{max}. Products A and B appear to be absorbed to the same extent on the basis of very similar values for AUC. However, product C is obviously less completely absorbed, as shown by the lower AUC.

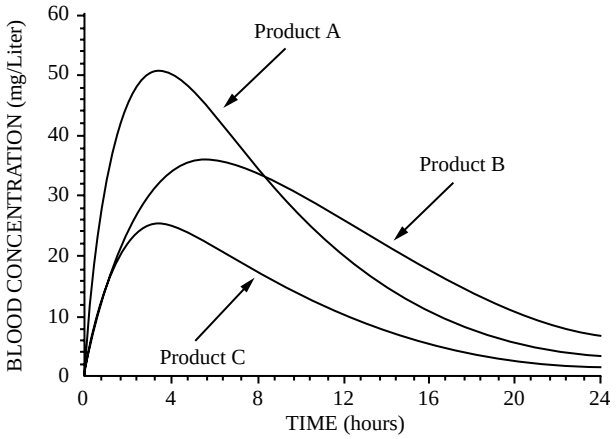

Fig. 2 Drug concentration in blood versus time after single doses of product A (rapidly and completely absorbed), product B (slowly and completely absorbed), and product C (rapidly absorbed, but only 50% as completely absorbed as products A and B).

All of the foregoing discussion assumes the absorption and elimination of the drug do not exhibit dose-dependent pharmacokinetics. For example, if the metabolism of a drug is a saturable process, the total AUC may be significantly increased for a rapidly absorbed dosage form because the initial blood drug concentrations exceed the metabolic capacity of the body to eliminate the drug. Nonlinear pharmacokinetics may also be caused by changes in the clearance of a drug due to factors such as nonlinear drug binding to plasma and/or tissue proteins. Thus, for a drug such as disopyramide, the AUC does not increase in proportion to the administered dose (7, 8). This is the result of a decrease in drug binding as drug concentration increases. Thus, it is necessary to measure free drug concentration in the plasma, rather than total drug concentration (free + bound), for disopyramide bioequivalence studies. Other mechanisms that can contribute to nonlinear pharmacokinetics and complicate the interpretation of bioequivalence data have been reviewed by Tozer and Tang-Liu (9). The interpretation of non-superimposable blood concentration time profiles for drugs that exhibit nonlinearity must be done with caution. Further, bioavailability and bioequivalence studies of such drugs may need to include multiple-dose protocols to further define the effect of differences in the rate and extent of absorption on the steady-state drug concentrations.

Urinary excretion studies

The estimation of bioavailability on the basis of the appearance of drugs in the urine is an attractive alternative to blood sampling because it represents a noninvasive method. This approach is particularly useful for drugs that have the urine as the site of activity (e.g., urinary tract antiseptics, such as nitrofurantoin and methenamine). This method is also useful for drugs that are extensively excreted unmetabolized in the urine, such as certain thiazide diuretics and sulfonamides. Often a less sensitive analytic method is required for urine concentrations compared with blood concentrations. If the urine concentrations are low, assaying larger sample volumes is relatively easy. The primary disadvantage to urinary excretion studies is that they require the collection of samples for a longer period of time to ensure the complete recovery of the absorbed drug. In addition, the subjects must be careful to completely void at each collection time and to avoid accidentally discarding any samples. As with the assessment of bioavailability from blood levels, urinary excretion studies also generally assume the pharmacokinetics are not dose dependent. If renal excretion is a saturable process, the percentage of the

drug excreted unmetabolized in the urine may not reflect the rate and extent of the drug absorption.

The three major parameters examined in urinary excretion bioavailability studies are: 1) the cumulative amount of drug excreted unmetabolized in the urine (ΣXu); 2) the maximum urinary excretion rate (ER_{max}); and 3) the time of maximum excretion rate (T_{max}). In simple pharmacokinetic models, the rate of appearance of drug in the urine is proportional to the concentration of drug in the systemic circulation. Thus, the values for T_{max} and ER_{max} for urine studies are analogous to the T_{max} and C_{max} values derived from blood level studies. The value of T_{max} decreases as the absorption rate of the drug increases, and ER_{max} increases as the rate and/or the extent of absorption increases. The value for ΣXu is related to the AUC and increases as the extent of absorption increases.

The calculation of excretion rate (ER) is based on Eq. 4

$$ER = \left(\sum Xu_2 - \sum Xu_1\right)(t_2 - t_1) \tag{4}$$

where ΣXu_1 and ΣXu_2 represent the cumulative amount of drug recovered in the urine samples obtained at sampling times up to t_1 and t_2, respectively. When constructing a plot of ER versus time, or for the determination of ER_{max}, the values for time are taken to be the midpoint of the urine collection period, that is, the midpoint between t_1 and t_2. Thus, estimates of T_{max} and ER_{max} from urinary excretion data provide less information on the rate of drug absorption than can be obtained from analysis of the blood concentration time profile, largely due to the fact that there is a limit to the frequency at which urine can be readily collected.

When sufficient urine samples have been collected to ensure that no significant amount of drug remains to be excreted, the cumulative urinary recovery is symbolized as $\Sigma Xu\ \infty$. The relative extent of absorption of drug 5 from two dosage forms may then be expressed as the ratio of the $\Sigma Xu\ \infty$ values. However, the value of $\Sigma Xu\ \infty$ is a function of the fraction (F) of administered dose (D) absorbed, the renal elimination rate constant (ke), and the rate constant for overall elimination (K) from the systemic circulation, as expressed in Eq. 5:

$$\sum Xu\omega = F * D * ke/K \tag{5}$$

Assay of other biological material

For a few drugs such as theophylline, saliva drug concentrations have been employed to supplement the collection of blood samples. However, the intersubject and intrasubject variability in saliva/plasma ratios have generally precluded the sole use of saliva drug concentrations to assess bioavailability. For some drugs such as cephalosporin antibiotics, clinical studies may also include a determination of the appearance of the drug in other body fluids, such as the cerebrospinal fluid and bile.

One might initially think that assessing the extent of drug absorption after oral administration would be possible by simply quantitating the amount of drug excreted in the feces. Such determinations occasionally provide useful data. For example, if subjects receive the drug as an enteric-coated tablet or some other solid dosage form and the product is recovered intact in the feces, there is little question regarding the lack of bioavailability. However, the data obtained from fecal recovery studies must be carefully interpreted. If the drug is analytically measured in a fecal sample, this does not establish that the drug was not absorbed. For example, certain drugs undergo extensive enterohepatic recycling and/or excretion in the saliva. Thus, a drug could be fully bioavailable, and, yet, a portion of the administered dose could be found in the feces. Further, the absence of intact drug in the feces is not proof of absorption because the drug may be degraded during its transit through the gastrointestinal tract.

Assessment of bioavailability from pharmacologic response

Topical application of a corticosteroid does not generally result in measurable blood concentrations of the drug. Thus, bioavailability and bioequivalence determinations for these drug products may involve measurement of dermatologic vasoconstriction (i.e., skin blanching), a pharmacodynamic response. A few studies have attempted to relate quantitatively a pharmacologic response to the oral bioavailability of a drug. For example, a relationship between the extent and duration of serum glucose concentration reduction and thez bioavailability of two dosage forms of tolbutamide was demonstrated (10, 11). Others have employed pharmacologic end points that were not necessarily related to the therapeutic activity of the test drug. For example, attempts have been made to relate pharmacologic responses, such as changes in pupil diameter, electrocardiogram readings, or electroencephalogram readings, to the time course of a given drug in humans and animals. However, pharmacologic data tend to be more variable, and demonstrating a good correlation between the measured response and the amount of drug available from the dosage form may be difficult. Further, the potential exists that the measured response may be due to a metabolite whose concentration is not proportional to the concentration of the parent drug responsible for therapeutic activity.

Assessment of bioavailability from therapeutic response

Because the ultimate goal of drug therapy is to achieve some therapeutic response in a patient, ideally the assessment of drug product efficacy should be studied in patients requiring the drug. Nonetheless there are good reasons to utilize healthy volunteers rather than patients. First, the quantitation of patient clinical response is too imprecise to permit a reasonable estimation of the relative bioavailability of two dosage forms of the same drug. Second, bioequivalence studies are usually conducted using a crossover design in which each subject receives each of the test dosage forms, and it is assumed that the physiologic status of the subject does not change significantly over the duration of the study. If patients, however, were utilized, this could be an invalid assumption because of changes in a patient's disease state. Third, unless multiple-dose protocols were employed, a patient who required the drug for a disease would be able to receive only a single dose of the drug every few days or perhaps each week. To avoid such problems, one could test each product in different groups of patients, but this would require both the use of a large number of patients and careful matching of the various patient groups. Fourth, many patients receive more than one drug, and the results obtained from a bioavailability study could be compromised because of a drug–drug interaction. Finally, an ethical question would arise in the case in which a particular product was believed to be defective. Thus, a patient requiring treatment with a given drug would need to consent to receive a product that might not provide sufficient drug for adequate treatment. Because of these considerations, the general conclusion is that most bioequivalence studies should be carried out with healthy subjects. However, for drugs that are not designed to be absorbed into the systemic circulation and are active at the site of administration, clinical studies in patients are the only means to determine bioequivalence. Such studies are usually conducted using a parallel, rather than a crossover, design. Examples include studies of topical antifungal agents, drugs used in the treatment of acne, and agents such as sucralfate used in ulcer therapy.

Other experimental approaches

The current bioequivalence regulations of the U.S. FDA describe several types of experimental approaches that do not involve human testing (3).

Use of experimental animals: Animal studies are not acceptable for bioequivalence determinations unless the data obtained with animals have been correlated with the data obtained in human studies, ensuring that the bioavailability of a dosage form in animals is closely related to that in humans. Animals are known to differ from humans in terms of gastrointestinal tract characteristics, metabolism, distribution, and excretion. For the study of solid dosage forms, relatively large animals, such as, dogs or monkeys, must be employed. Although such studies can provide useful data during dosage form development and may serve as an alternative to human subjects for drugs that are quite toxic to humans (e.g., cancer drugs), in general, animal studies are not acceptable as the final assessment of the bioavailability of a dosage form.

In vitro methods: In recent years, there has been great interest in the development of laboratory test systems that can simulate the disintegration and dissolution of a drug product in the human gastrointestinal tract. The development of such devices is to reduce the need for human testing. One of the early approaches to relate in vivo bioavailability data to in vitro measurements employed testing based on the time required for a solid dosage form to disintegrate in a particular solvent. The official apparatus employed for such testing is described in the U.S. Pharmacopoeia XXIV (USP) (12). However, the problem with this method is that the measurement of the time required for a dosage form to break into small particles may not necessarily relate to the dissolution rate of the drug. The current XXIV describes one official in vitro disintegration apparatus (i.e., basket-rack assembly) and two official dissolution apparatus (i.e., one with a paddle and one with a basket stirring element) for the evaluation of solid dosage forms (12). Although these methods are well-established and used extensively, few in vitro/in vivo correlations between dissolution data and human bioavailability data have been established. In vitro dissolution testing is useful as a standard for monitoring product quality and for distinguishing between dosage forms for which a bioavailability problem is known to exist. However, the USP acknowledges that many of the formulation factors that affect the performance of a drug product during in vitro dissolution testing may only sometimes affect the in vivo bioavailability of the drug (i.e., dissolution testing may identify subtle differences in the characteristics of the dosage form that are not relevant to its in vivo performance) (12). Thus, in vitro dissolution testing cannot be assumed to relate to the in vivo bioavailability of a given dosage form.

In conducting dissolution studies, the choice of an appropriate solvent is very important. Dissolution experiments should be conducted using conditions that mimic the environment in the gastrointestinal tract. Typical dissolution studies employ 0.1 *N* hydrochloric acid, water, or a buffer as the dissolution media. Simulated gastric

fluid (pH 1.2), with or without pepsin and simulated intestinal fluid (pH 6.8), with or without pancreatin, are also commonly employed for in vitro dissolution testing. It is widely recognized that the dissolution of controlled-release dosage forms may be pH-dependent. Thus, the US FDA recommends that dissolution testing for controlled-release dosage forms be conducted over a wide range of pH values, including 1–1.5, 4–4.5, 6–6.5, and 7–7.5, with multiple time determinations to better characterize the dissolution properties of the dosage form (1–3). Bioequivalence determinations for products containing cholestyramine resin (used to control cholesterol) represent a novel in vitro approach to bioequivalence testing. Generic versions of such drug products are evaluated in vitro by determining the rate and extent of the interaction of different bile salts with the resin.

Experimental Design

The proper design of a bioavailability or bioequivalence study is essential to the collection of meaningful data. Studies must include a sufficient number of subjects, and blood or urine samples must be collected at appropriately spaced intervals to characterize accurately the pharmacokinetics of the drug product(s) and make statistically relevant conclusions regarding its bioavailability and bioequivalence. Amongst other factors, study design must consider the characteristics of the study population (e.g., age, weight, gender, race, and health), the timing of dose administration, meals, and blood sample collection, and the time interval (i.e., washout period) between consecutive administrations of the drug products. In a bioequivalence study involving two or more dosage forms, the sequence of product administration must also be carefully considered to minimize experimental bias. The purpose of these rigorous controls on experimental design and conduct is to minimize the variability associated with pharmacokinetic (e.g., clearance, volume of distribution, and absorption) and physiologic (e.g., gastric emptying and pH) factors, such that the variability observed during the study is more closely related to the performance of the drug product(s) under consideration.

Crossover designs versus other designs

The most common type of study uses a crossover design in which each subject receives each of the test products. In such a design, differences among dosage forms, subjects, and sequences of administration can be readily estimated. In essence, each subject serves as his own control. Crossover designs have also been developed to minimize the effects of residual or carryover effects, which could occur if the administration of a given dosage form had an influence on the bioavailability of a subsequently administered product. Table 2 illustrates a crossover design that could be employed to evaluate the relative bioavailability of three dosage forms (A, B, and C) in a group of 12 human subjects. Note that there are six possible sequences for the administration of each of the three products. Further, each subject receives each of the three products, and each dosing period contains all three products. The value of such a design is that it minimizes any bias relating to subject and dosing sequence effects. Replicate study designs, in which each subject receives the test and reference drug products on more than one occasion, are currently being evaluated as an alternative method to examine the bioequivalence of drug products.

Table 2 Three-way crossover design for bioequivalence study

	Dosing period:		
Subject	**Period 1**	**Period 2**	**Period 3**
1 and 2	A	B	C
3 and 4	A	C	B
5 and 6	B	A	C
7 and 8	B	C	A
9 and 10	C	A	B
11 and 12	C	B	A

Single-dose versus multiple-dose studies

Bioavailability studies intended to determine the disposition of a drug, particularly those involving new chemical entities, must include both single- and multiple-dose administration. However, most bioequivalence studies, which compare the bioavailability of two or more dosage forms, usually employ only single-dose administration for each product, under fasting conditions. One major exception is bioequivalence studies of controlled-release products. The U.S. FDA requires both single- and multiple-dose administration, as well as a determination of the effect of food on the absorption of the drug from the dosage form (1–3). However, the requirement of multiple dose studies in assessing the bioequivalence of controlled-release products while receiving much attention recently, may be abandoned, with the thought that single dose studies provide more sensitive information to assess the performance of these products.

Multiple-dose studies are more difficult to control, and they expose the subject to more drug. However, multiple-dose study designs also have advantages. They are more representative of how drug products are usually used by patients, and they also require fewer blood

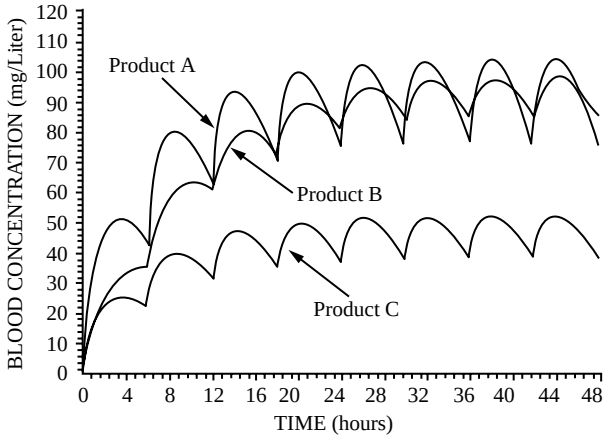

Fig. 3 Drug concentrations in blood vs. time after multiple doses of three products administered every 6h. The products (A, B, and C) are the same as those illustrated in Fig. 2.

samples and less sensitive analytical methods. Such studies require a sufficient number of doses to permit the achievement of steady state, which may be defined as the point where the amount of drug being absorbed into the body is equal to the amount being eliminated from the body. A general rule is that dosing must continue for approximately five biological half-lives in order to be within approximately 95% of steady state. Once steady state has been reached, the area under the blood concentration time curve during a single dosing interval should be equal to the value of the AUC $(0-\infty)$ from a single dose (assuming dose-independent clearance of the drug). Thus, it is necessary to obtain blood samples over only a single dosing interval at steady state in order to determine the AUC. The dosing interval selected for sampling (e.g., 7 A.M. through 7 P.M. at steady state, if dosing occurs every 12 h) should be identical for each study phase. Because the disposition of the drug could vary as a function of time of day, comparing an AUC determined during the period 7 A.M. through 7 P.M. for one product and the AUC determined from 7 P.M. through 7 A.M. for a second product would not be valid.

An example of the results of a steady-state study, with dosing every 6 h, is illustrated in Fig. 3. The pharmacokinetic data employed to generate the results shown in Fig. 3 were identical to those used for Fig. 2. The results demonstrate the influence of the rate and extent of absorption on the steady-state plasma concentrations. The lower plasma concentrations shown for product C reflect the lower extent of absorption for this product. Products A and B have the same extent of absorption but differ in rate of absorption. Product A is more rapidly absorbed than product B, and, thus, there is a greater fluctuation between the maximum and minimum concentrations at steady state.

Other study design considerations

In the design or evaluation of the results of a bioavailability or bioequivalence study, it is important to establish that an adequate number of subjects were studied and that an adequate number of blood and/or urine samples were collected. As in most scientific studies, the use of too few subjects precludes reaching meaningful decisions regarding the significance of differences that may be observed. Moreover, values for T_{max}, C_{max}, orER_{max} cannot be accurately determined if the time interval between samples is too great. Further, accurate estimates of AUC and/or total urinary recovery of drug are not possible if blood and/or urine collections are terminated prematurely. As a general rule, collecting blood samples for at least two to three biological half-lives is desirable, and urinary excretion studies should include urine collections for five to seven biologic half-lives. Some extension of these guidelines may be necessary if the study involves controlled-release dosage forms that may result in absorption for a prolonged period. If data are not available for the half-life of a drug, a reasonable guideline is to continue blood sampling until blood concentrations have declined to less than 10% of the peak concentrations. Similarly, urine collections should be continued until significant quantities of drug are no longer being excreted. A typical bioavailability study utilizes from 12 to 24 or more healthy subjects who have no history of any disease that could affect the disposition of the test drug. The subjects are required to refrain from taking any drug other than the test compound for a period (usually one week or more) prior to the study and throughout the course of the study. Certain drugs, such as, enzyme inducers, could affect the disposition of the test drug. Further, depending on the specificity of the assay, other drugs may interfere in the quantitation of the drug of interest. Usually, only subjects between the ages of 18 and 40 are employed, unless there are specific reasons for employing other subject populations. Both male and female subjects should be included in the study, as well as subjects of differing race, realizing, of course, that the heterogeneity that one may accommodate in a study of 12 subjects is limited if statistically meaningful comparisons are to be made between the groups. Finally, for typical single-dose studies, the subjects are required to fast from approximately 12 h before the dose until 4 h after the dose, unless the objective of the study is to determine the influence of food on absorption of the drug.

Analytical methodology

One of the most important considerations in any bioavailability study is the validity of the analytical

method. In addition to being reproducible, it must be sufficiently sensitive to permit the detection of low drug concentrations in the biologic sample. After single-dose administration, drug concentrations in plasma are frequently in the low microgram or nanogram per milliliter range. Further, the method must be specific for unmetabolized drug and be capable of accurately determining drug concentrations in the presence of metabolites of the drug and the constituents of blood and/or urine. Most analytical methods involve some type of clean-up step, such as, solvent extraction, to separate the drug from the biological fluid. In addition, most assays employ some type of chromatography, often using either gas chromatography or high-performance liquid chromatography. Assay validation is a critical component of any bioavailability or bioequivalence study and should consider the accuracy, precision, sensitivity, specificity, linearity, and reproducibility of the analytical method. In the biologic sample, the stability of the drug during storage must also be given careful consideration.

Analysis and Interpretation of Data

Bioequivalence studies are usually intended to demonstrate that two or more formulations do not differ significantly (i.e., that the products are therapeutically equivalent and interchangeable). Once the data from a bioequivalence study are collected, statistical methods must be applied to determine the level of significance of any observed differences. Statistical comparisons of the C_{max} and AUC are performed after log transformation. Log transformation is appropriate because 1) many biological and pharmacokinetic parameters are log-normally distributed, and 2) it is the ratio of C_{max} and AUC between drug products, and not the absolute difference between the mean values, that is most relevant for comparison. Also, one-sided statistical tests at a 0.05 level of significance are performed using the log-transformed data from the bioequivalence study. It is important to note that 90% confidence intervals (CIs), and not the mean values, of C_{max} and AUC are employed to make this comparison and assure the bioequivalence of the drug products. In other words, the 90% CI for the mean C_{max} and mean AUC for the drug product must be between 80 and 125% of the respective mean values for the reference dosage form (e.g., a drug product with a mean C_{max} of 83% of the reference product with a 90% CI of 79–87% would not be considered bioequivalent). In fact, if the mean C_{max} or mean AUC of the drug product differs by more than 10–15%, the CI is likely to fall outside the 80–125% range. Typically, an analysis of variance (ANOVA) method appropriate for the study design is applied to evaluate differences among dosage forms, subjects, and treatment periods. Mean T_{max} values are also computed, but unless large differences are observed, they are generally not used for the bioequivalence determination, largely due to the fact that T_{max} values are highly dependent on study design and the time at which blood samples were collected.

SUMMARY

The measurement of the bioavailability and bioequivalence of drug dosage forms is commonplace. The numerous reasons for the increased use of such studies include: 1) rapid growth of the generic drug industry; 2) an increased awareness of the effect of dosage form on the rate and extent of drug absorption; 3) the development of more sensitive and reliable analytic methods to quantitate drugs and metabolites in the body; and 4) the need to have a means to evaluate the vivo performance of a dosage form by a means other than clinical trials in patients. Clearly, bioavailability and bioequivalence studies have become a routine part of the drug product approval process by governmental agencies. Such studies provide valuable information regarding the disposition of a drug in humans and the factors that may influence its performance. These data are useful in the development of new dosage forms and assist in the preparation of appropriate labeling for a drug product. Finally, bioequivalence studies have become an essential part of the comparison of pharmaceutically equivalent products manufactured by different pharmaceutical firms.

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